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This document summarizes the physical/chemical properties, toxicological information and environmental fate and effects of Benzidine, with emphasis on its aquatic behavior. From these data criteria are developed for the protection of aquatic life.

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CRITERIA DOCUMENTS FOR TOXIC POLLUTANTS

Scientific rationale and criteria developed pursuant to Section 307(a) of the Federal Water Pollution Control Act, P.L. 92-500, 33 U.S.C. §§ 1251 et seq., (1972), for the development and establishment of effluent limitations for toxic substances are set forth in the following chapters.

Section 307(a)(2) states inter alia that a proposed effluent standard "...shall take into account the toxicity of the pollutant, its persistence, degradability, the usual or potential presence of the affected organisms in any water, the importance of the affected organisms and the nature and extent of the effect of the toxic pollutant on such organisms..." Thereafter, having considered these factors, the Administrator is to set an effluent standard for toxic pollutants which provides an ample margin of safety.

In the development of criteria which serve as both the basis and the goal for the establishment of effluent limits, reliance was placed on the toxicity data derived from laboratory studies on a range of organisms including invertebrate, vertebrate, and mammalian test species. These studies provided extensive acute and chronic toxicity data based on feeding experiments for a wide range of aquatic organisms and consumers of aquatic organisms. Environmental studies documenting bioaccumulation in the food web of the toxic material by the food chain organisms and bioconcentration by organisms directly from water provided an important component data base upon which criteria were derived. Appropriate human toxicity data and mammalian carcinogenic studies, where available, were used also in developing criteria.

Aquatic toxicity data generally are obtained by one of two basic methods, the static and flow-through bioassay. The more traditional static bioassay employs a tank in which the test organisms are living and to which a given concentraction of toxicant is added. Any water loss due to evaporation is made up by the addition of fresh water. The flow-through bioassay, which is a more recent development, reflects more nearly the natural conditions. Concentrations of toxic substances are constantly maintained and provide a more accurate test of sensitivity of aquatic species. Water in a flow-through test is replenished constantly through flushing. Comparative results using the static and flow-through bioassays demonstrate that flow-through data yield lower toxicity values for a pollutant than a static bioassay. This fact is demonstrated by comparative studies as discussed in the endrin document. However, most of the data available were developed using static bioassays.

Some toxic pollutants are extremely stable and degrade only slowly or form persistent degradation products. Those pollutants which degrade rapidly pose a less severe long-term hazard unless their entry to the environment is continuous. A parent compound, e.g., aldrin, may rapidly degrade or be altered to a more toxic form, i.e., dieldrin.

Bioconcentration of toxic pollutants is a significant consideration in the development of criterion. The rate and degree of accumulation in an animal and the rate of loss from the animal are factors that help define the potential magnitude of the pollution load problem. As an

example, a pollutant which bioaccumulates presents a hazard both to aquatic systems and potentially to man or other carnivores associated with the ecosystem. To satisfactorily manage a persistent or non-degradable pollutant requires the maintenance of a ceiling for ambient levels in water which will afford protection to the food chain and the consumers of aquatic life (animals including humans). The body burden of toxic pollutants in fish or food chain organisms may have no outward effect on the species but will affect consumers of that food level. As an example, the brown pelican, when feeding on endrin-contaminate fish may die or suffer species depletion through reproductive impairment.

Data on toxic effects of pollutants are not available for all species that may be exposed to the toxic pollutant in these complex societies. Such data would be necessary to ensure protection of the most sensitive species. It is desirable to know the relative sensitivity of a wide variety of species in order to have a better estimate of the sensitivity of the untested, most sensitive species. Because such data are not available on all species, the range in sensitivity of a small number of tested species is used to provide a measure of the range of sensitivity of all species.

The natural aquatic environment includes many kinds and life stages of plants and animals that are intricately interrelated to form communities. Criteria are developed to protect these interrelationships and incorporate aquatic toxicity data for a phylogenetic cross section of organisms as well as

species representative of wide geographic distribution. Chronic studies are an important consideration in establishing criteria and require studies of at least one generation, i.e., one reproductive cycle. Use of an application factor for persistent and bioaccumulated toxic pollutants represents consideration of a safety factor. As discussed in the National Academy of Science publication on water quality (p. 185 of the NAS/NAE Water Quality Criteria -- 1972, GPO-5501-00520), the use of an application factor of 0.01 when applied to acute toxic values is thought to provide an ample margin of safety for certain chlorinated hydrocarbon pesticides.

Ecological importance of an organism is dependent on the role the organism plays within the ecosystem and upon its relationship to the food chain within the aquatic community and to consumers of aquatic life, including man. Thus, toxicity data for the top carnivores in a given ecosystem, as well as economically important species such as trout, salmon, menhaden and shrimp are needed for the development of a protective criteria level. Toxicity data for organisms such as the stonefly and Daphnia are of equivalent importance since these organisms are a food base for higher consumers and are representative of invertebrate species found in most waters of the United States.

Invertebrate species, such as the stonefly and the Daphnia, are an indication of the integrity of the aquatic food chain and their presence may be the controlling factor for the abundance of economically and

recreationally important predators such as trout, bass or pike. While these fish may not directly consume the Daphnia or stonefly or, in fact, even inhabit the same waters, these lower order organisms are representative of the food chain base supporting predators.

Criteria levels, by their nature, are developed to protect aquatic organisms and consumers of aquatic life from direct toxic effect when placed on contact with the toxic pollutants; and, to protect from a more insidious and even greater danger, e.g., chronic effects. Chronic effects take the form of reproductive failures or the poisoning of predators consuming food organisms which have bioaccumulated levels of toxic pollutants as in the case of the brown pelican and consuming endrin loaded fish (see Attachment D, Endrin), and a variety of other physiological effects as discussed in the various documents. Decreases in aquatic organisms or consumers of aquatic life not always are coupled to point source discharges of toxic pollutants at concentrations below acute toxic levels; however, the addition of toxic levels which are not acutely toxic can achieve the destruction or at least disruption of aquatic systems by causing reproduction of failure. Hence, the need for application factors. The relationships between discharges of toxic pollutants and effects on important organisms of economic and environmental importance and consumers of these organisms are well documented in the criteria documents.

BENZIDINE

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I. Preamble

Benzidine is a proven human carcinogen with the site of tumor induction being the bladder. Therefore, in deriving an ambient water quality criteria considerations beyond the aquatic toxicity are necessary. As calculated in Appendix 1, levels of benzidine which could result in a cancer risk of no more than one case per million people exposed, at the 95 percent confidence limit, during a lifetime, if allowed to reach man through water, are projected to ranges from 0.05 ug/l to 1.0 ug/l. A level of 0.1 ug/l is recommended based on the calculated dose/response data, the use of extrapolative methods to determine a risk level, and the possible population at risk.

Benzidine has not been studied extensively for toxicity to aquatic species since the main concern has been its carcinogenic activity in man. However, initial bioassays with benzidine have produced 96 hour TL_{50}'s of 2.5 to greater than 20.0 mg/l for fish. A bioaccumulation study with benzidine has shown that edible tissue residues can accumulate 44 times the ambient water level. Use of an 0.01 application factor applied to the 96 hour TL_{50} for fish results in a level of 25 ug/l which would protect aquatic life.

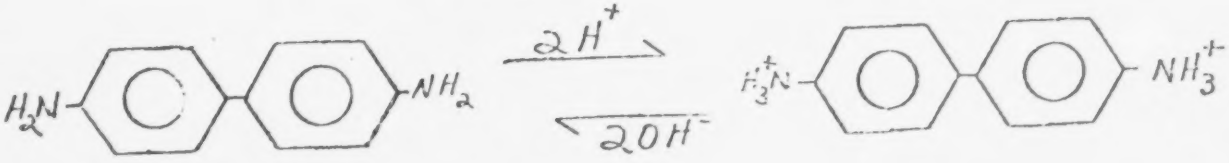
II. Physical and Chemical Properties

Benzidine (4,4'-diaminobiphenyl) is classified as an aromatic amine. It has the empirical formula $C_{12}H_{12}N_2$ and a

molecular weight of 184.24 grams (1). Benzidine exists as a grayish-yellow, white, or reddish-gray crystalline powder with a melting point of 128°C and a boiling point of 400°C at 740 mm mercury (2). Benzidine's solubilities in water range from 0.4 g/l at 12°C to 9.4 g/l at 100°C. Organic solvents increase the solubility of benzidine with 20 g/l dissolving in ethyl ether and 200 g/l in absolute ethanol (3).

As an aromatic amine, benzidine has an inherent basicity. Although amines are much weaker bases than hydroxide ion or ethoxide ion, they are much stronger bases than water. Aqueous mineral acids or carboxylic acids readily convert amines into their salts; aqueous hydroxide ion readily converts the salt back into free amines. As seen in figure 1, benzidine is easily converted to and from its salt (4).

Figure 1



Benzidine

Benzidine Salt

Benzidine can readily undergo diazotization reactions. This ability to undergo diazotization makes benzidine suited as a basic building block for dyes. Diazotization is the reaction of a primary aromatic amine with nitrous acid in the presence of excess mineral acid to produce an azo (-N≡N-) compound (24).

The azo compounds are strongly colored, with the color depending upon the exact structure of the molecule. Because of their color, the azo compounds are of importance as dyes; about half of the dyes in industrial use today are azo dyes (4).

III. Toxicological Data

A) Invertebrates

Benzidine's toxicity to invertebrates was investigated by Lemke in 1973 (18). Toxicity data for the scud, Gammarus pseudolimnaeus, was obtained through a static bioassay. The 96-hour TL_{50} value was found to be +20 mg/l.

B) Fish

Benzidine has not been studied extensively for toxicity to aquatic species since the main concern has been its

carcinogenic activity in man. However, initial bioassays with benzidine have shown that it is not highly toxic to fish.

Lemke in 1973 (18) determined 96-hour TL_{50}'s for five species of fish. Toxicity data for these five species are shown in Table 1.

Table 1 (18)

TL_{50} Values for Static Bioassay with Benzidine

Species	TL_{50} Values (mg/l) 24 hr.	48 hr.	72 hr.	96 hr.
Flagfish (*Jordanella floridae*)	+50	32.5	25	16.2
Fathead Minnows (*Pimephales promelas*)	+20	+20	+20	+20
Red Shiner (*Notropis lutrensis*)	+20	10	2.5	2.5
Lake Trout (*Salvelinus namaycush*)	8.7	5.0	4.35	4.35
Rainbow Trout (*Salmo gairdneri*)	+20	14.1	10	7.4

The Synthetic Organic Chemical Manufacturer's Association (SOCMA) in 1975 (19) determined TL_{50} values for three different species of fish. Their results are listed as Table 2.

Table 2 (19)

96-hour TL_{50} Values for Benzidine

Species	96-hour TL_{50} Value (mg/l)
Fathead Minnows (Pimephales promelas)	2
Emerald Shiners (Notropis atherinoides)	5
Bluegills (Lepomis macrochirus)	15

C) Mammals

The history of cancer research shows that the discoveries in man of carcinogenesis by various chemicals preceded studies of these substances in experimental animals. Such was the case with benzidine. Animal studies were used to gain more information about the carcinogenic action of benzidine.

As early as 1895, Rehn (20) related certain bladder tumors to exposure to aromatic amines. However, isolation of the compound or compounds responsible for bladder tumors was difficult since many of the workers studied were exposed to an array of compounds. Through selective research the major suspected carcinogens were identified by Barsotti and Vigliani in 1949 (21) as beta naphthylamine and benzidine.

Early attempts to induce experimental tumors in animals with benzidine had been without success (22, 23), and it was not until 1950 that Spitz et al. (7) demonstrated that benzidine was carcinogenic. Tumors occurred in the liver, intestine, and the acousti gland in 20 percent of the test rats injected with a maximum cumulated amount of 1.28g of technical-grade benzidine. Purified benzidine and benzidine sulfate were also carcinogenic at a similar dosage level (total dose: 0.96 and 0.94g, respectively). However, no

tumors occurred in either rabbits or monkeys after injection of benizidine. Only one case of bladder cancer was found in dogs fed a total dose of 325g of benzidine in five years. No conclusions concerning the carcinogenicity of benzidine for dogs was drawn from this single occurrence.

1) Biochemistry of Benzidine

The failure to obtain bladder tumors with benzidine in previous experiments was thought to be due to the species of the animals used, the mode of administration of the chemical, or the degree of dosage. But, after Spitz's successful experiment, the importance of the metabolism of benzidine was recognized. Studies were conducted to determine the fate of benzidine upon entering the body.

Work by Weber and Heideprim in 1928 (9) and Goldblatt in 1947 (8) indicated that benzidine may be oxidized <u>in vivo</u>. Baker in 1950 (10) demonstrated that oxidation may take place to give 3,3'-dihydroxybenzidine. Further work by Baker and Deighton in 1953 (11) determined the rate of recovery of benzidine from tissue and the extent of conjugation of the aromatic amino groups. Their studies showed that 93 percent of the injected dose was recovered as diazotizable material after 4 hours and 68 percent after 12

hours. Of this, the conjugated form accounted for 24 percent and 49 percent respectively of the recovered amount.

Sciarini in 1957 (12) identified free benzidine and the metabolite 3-hydroxybenzidine in the urine of dogs following intraperitoneal injection of benzidine. Troll and Nelson in 1958 (13) found the major metabolites of benzidine in dogs, rabbits, and guinea pigs to be the sulfate and glucuromide of 3- hydroxybenzidine.

Sciarini and Meigs in 1958 (14) verified the metabolites and quantified the constituents in the dog. As shown in Table 3, only about 2 percent to 9 percent of unmetabolized benzidine was recovered in dog's urine.

Table 3

Benzidine Recovery from Dog Urine Following Intraperitoneal Injection at Different Dosage Levels

Dose Administered (mg/kg Body Wt)	Percent Free Benzidine Recovered in 24 hours
100	6.8
56	4.0
25	1.7
9	3.9
2.0	9.0
1.0	2.7
0.5	3.2
0.25	6.4
0.2	4.0
0.1	7.0
0.05	2.0

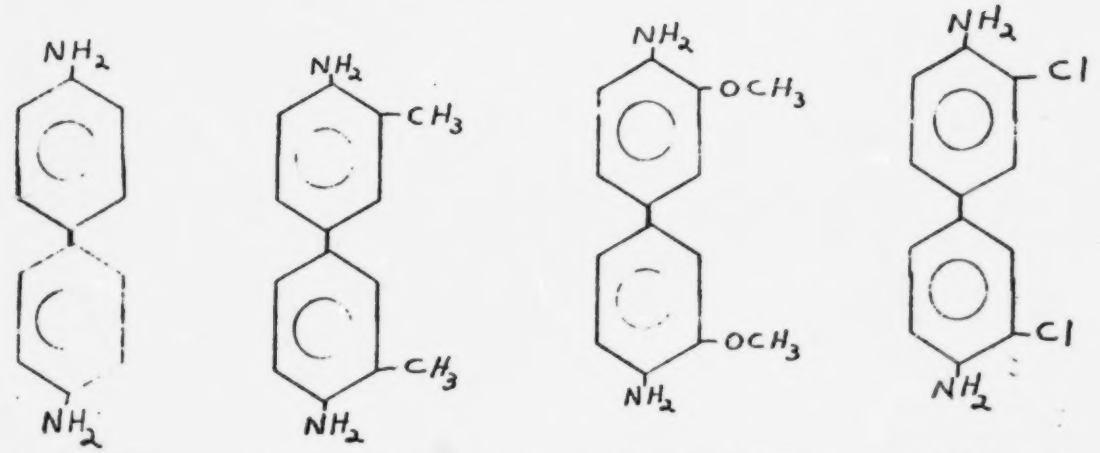
The average free benzidine recovered amounted to about 7 percent of the dose administered while the major metabolite (3-hydroxybenzidine) was approximately 47 percent of the dose. The feces of the dog were found to contain about 10

percent as much benzidine and its metabolite as detected in the urine.

Subsequent investigations by Sciarini and Meigs in 1961 (15) identified other metabolites in mice and benzidine workers. The recovery and identification of only a portion of the dose of benzidine administered to the mice was consistent with the hypothesis that additional metabolites were present. Through analysis of the urine of workers engaged in benzidine manufacture, four substances were identified in the following proportions: free benzidine 3.6-5.6 percent; monoacetylbenzidine 1.6-5.4 percent; diacetylbenzidine 5.1-10 percent; and 3-hydroxybenzidine 78.5-89.7%.

Experiments with substituted benzidines conducted by Sciarini and Meigs in 1961 (16) were designed to broaden the understanding of the behavior of benzidine. The three disubstituted benzidines used were: dicrthotolidine, dianisidine, and dichlorobenzidine. Structures of benzidine and the disubstituted benzidines are shown in figure 2.

Figure 2



Benzidine Diorthotolidine Dianisidine Dichlorobenzidine

Both dianisidine and dichlorobenzidine were found to metabolize in the human body along different pathways than benzidine. Diorthotolidine follows a metabolic route similar to that of benzidine.

Neumann's study in 1970 (17) of recognized carcinogenic aromatic amines demonstrated that it is the metabolites or reactive intermediate stages of benzidine formed during metabolism which are considered to be the carcinogenic substances.

However, even products such as azo dyes, which are derived from benzidine, have been shown to undergo metabolic reduction to free benzidine. Rinde and Troll (63) in 1975, reported on the metabolic reduction of four benzidine azo

dyes in the rhesus monkey. By comparing the excretion of free benzidine after feeding the monkeys either benzidine or the dyes, it was determined that the metabolic reduction of the dyes to free benzidine was nearly complete. Yet, Rinde and Troll stipulate that they do not intend to imply that all azo dyes are biologically reduced to carcinogens, but those derived from carcinogenic amines should receive particular attention.

2) Physiological Effects

Concurrent with the investigation of the metabolic pathways of benzidine, studies were undertaken to experimentally produce tumors in mammals through exposure to benzidine.

Boyland et al., in 1954 (25) fed female Wistar rats a diet containing 0.017 percent benzidine. Chlolagiomas and liver-cell tumors were reported, but urinary bladder carcinomas were not found.

Bonser et al., (26) injected benzidine into mice, subcutaneously at a weekly dose of 6mg for 52 weeks. Hepatomas and lymphomas occurred in the treated animals, while lymphomas occurred in one-third of the untreated control animals. Nevertheless, the presence of the hepatomas caused benzidine to be classified as carcinogenic.

Loumonier and Laquerriere in 1962 (27) studied the effect of daily applications of benzidine (3 percent in benzene) painted to the back skin of Wistar rats. After 15 days of treatment, jaundice appeared due to toxic hepatitis. After a latent period of 2-4 months, the rats developed malignant tumors, primarily hepatomata.

In 1964, Pliss (28) studied the tumorigenic properties of benzidine in rats. A total dose of 300mg of benzidine was subcutaneously injected during a 6 months period, however, there was a considerable loss of animals, especially females, during the first few months of the experiment due to acute toxic effects. Only 20 rats (40 percent), including 5 females survived for 5.5 months. The deceased animals showed profound degenerative changes in the liver with ultimate necrosis. Hepatomata, cymbal gland tumors, and sarcomata in the underlying fat at the site of injection of benzidine were reported in 70 percent of the surviving rats.

Saffiotti et al., (29) and Sellakumar et al., (30) investigated the effects of benzidine on Syrian golden hamsters. Benzidine was administered to hamsters in the diet at concentrations of 0.1 percent for their lifespan. Hepatomas, liver-cell and chloangiomatous tumors developed, but none appeared in the bladder.

Griswold et al., in 1968 (31) reported that benzidine produced breast cancer in female Sprague-Dawley rats after intragastric feeding ranging from 12 to 50 mg/rat during a 30 day period.

In 1970, Zabezhinskii (32) reported the results of benzidine inhalation experiments on rats. The animals were exposed to a concentration of benzidine in the air of 10-20 mg/cu m for 4 hours a day, 5 times per week, for 20 months. After inhaling benzidine for 13 months, 29 percent of the rats developed tumors consisting of myeloid leukemia, fibroadenomata, carcinomata of the mammary gland (male and female), and hepatoma.

The induction of hepatic tumors in mice by benzidine was reported by Prokofjeva in 1971 (33). Single weekly doses of 6 mg per animal were subcutaneously injected into 181 mice (C3HA line) over a period of 8-13 months (total dose: 210-336 mg). Of the 46 mice that survived for 15-28 months, 31 (67.4 percent) had developed hepatomata. A previous control colony of C3HA mice showed a 1 percent hepatoma frequency in untreated animals.

A study was conducted by Vesselinovitch, et al., reported in 1975 (71), to assess the carcinogenicity of benzidine hydrochloride in mice. In one carcinogenicity study, 200 six-week old B6C3F mice were given benzidine dihydrochloride intermittently by stomach intubation, twice weekly, in the amounts of 0.5 or 1.0 mg/mouse at each intubation for 84 weeks. All animals were killed at 90 weeks of age, at which time their tumor incidence was

evaluated. Benzidine-treated mice developed liver tumors, Harderian gland tumors, lung adenomas, and lymphoreticular tumors. A total of 124 of the indicated tumors were reported for both dose levels.

D) Human

The incidence of bladder tumors resulting from occupational exposure to aromatic amines was first described by Rehn in Germany in 1895 (20). Between 1905 and 1932, bladder tumors in dyestuff workers were reported in Switzerland (36), Great Britian (37), Russia (38), and Austria (39). The first cases of this condition in the United States were described by Ferguson in 1934 (40). Subsequently the disease has been recognized in Italy (41), Japan (42) and France (43).

The noted high occurrence of bladder tumors in the dye industry was an established fact, though the etiology of the disease was still obscure. Early theories implicated certain intermediate products used in the manufacture of dyes (44). Of these, aniline, benzidine, and alpha and beta naphthylamines were the most frequently implicated (45, 46, 47, 48).

In 1949, Barsotti and Vigliani (49) examined 186 workmen cystoscopically to follow the evolution of bladder lesions. Through their study, the following conclusions were made:

1) Aniline has no appreciable carcinogenic potential.

2) Benzidine and beta-naphthylamine have the highest carcinogenic potential among the aromatic amines studied.

Scott in 1952 (5) investigated the incidence of bladder tumors in an English dyestuffs factory. Sixty-six cases of bladder tumor were reported. Of the sixty-six cases there were 30 (23 in the manufacturing section and seven in the handling section) who were exposed to benzidine, and who had never been exposed to beta naphthylamine.

In 1954, Case et al., (50) published the results of their study of workmen engaged in the manufacture and use of aniline, benzidine, and alpha and beta-naphthylamines in the British chemical industry. They determined the incidence of tumors of the urinary bladder among such workers. The data indicated that the incidence of bladder cancer among persons exposed to benzidine greatly exceeded that among the general population. The study also showed bladder cancer to be a

fatal disease, only 20 percent of all cases having survived more than 10 years from the first detection of the disease.

By 1957, based upon the findings of Case et al., (50), the manufacture of benzidine had been accepted as an occupational hazard by the Association of British Chemical Manufacturers. That year, plant and operating recommendations were made by Scott and Williams (51) to ensure the safe manufacture and use of products causing tumors of the bladder.

Long term retrospective studies were undertaken in order to assemble additional information on the occurrence and natural history of malignant tumors in workers exposed to dyes and dye intermediates.

In 1962, Goldwater et al. (52) studied the incidence of urinary bladder tumors in workers exposed to alpha-naphthylamine, beta-naphthylamine and benzidine, to determine the average incubation period, the average survival time, and the incidence of malignant tumors other than those of the bladder. The population studied consisted of 366 male workers in a coal tar dye factory employed between 1912-1962. Of the 366 workers studied, 76 were exposed to benzidine alone. Bladder cancer developed in 17, or 21.3 percent, of the 76 exposed to benzidine. The induction of bladder cancer from benzidine had an average

incubation period of 18.7 years, calculated from the first exposure to diagnosis of malignancy. Details of the calculated incubation periods are given in Table 4.

Table 4

Bladder Tumors Related to Years of Exposure

		Years of Benzidine Exposure	
		Range	Mean
A)	First suggestive genitourinary complaints	9-30	20.7
B)	First positive cystoscopy	4-31	16.6
C)	First diagnosis of papilloma	4-28	18.4
D)	First diagnosis of malignancy	5-33	18.7

Following diagnosis of bladder malignancy in workers exposed to benzidine, the longest pericd of survival was ten years, the shortest was less than a year. The mean survival time between diagnosis of cancer and death was approximately 3 years.

Among the 366 men studied, there were 11 recorded malignant tumors other than cancer of the bladder, as follows:

Lung	3
Prostate	3
Stomach	2
Brain	2
Lymphoma	1

There was no evidence of any unusual incidence of malignancies other than those of the urinary bladder.

Rye et al., in 1970 (53) discussed the use of benzidine and its congeners, (diorthobenzidine, dianisidine, and diorthotolidine) as curing agents in polyurethane production. There was no evidence of cancer developing among the workers exposed during production of the three congeners. However, several bladder tumors had been found

in men working in a plant that alternated production of benzidine and its three congeners.

In 1973, Zavon et al. (54) published their findings associating the manufacture of benzidine with a high risk of developing bladder cancer. They observed a group of 25 workers engaged in manufacturing benzidine over a 13-year period. Of the 25 workers, three had about one year of exposure to beta-naphthylamine, three had exposure to orthotoluidine, and seven had been on dichlorobenzidine production at some time. The mean exposure to benzidine was 10.6 years.

Thirteen of the group (52 percent) developed bladder tumors, some multiple. The study group had an average induction time from first exposure to detection of the first tumor of 16.6 years. This time period agrees with previously calculated induction times (50, 52, 55, 56).

By 1970 only one member of the thirteen bladder cancer patients had died, which gives a ten-year survival of 92 percent. However, this survival period was expected to increase because no recurrences or life-threatening complications were present at that time. A mean survival of three years was reported by Goldwater et al. (52) for bladder cancer and the ten-year survival of less than 20

percent was reported by Case et al. (50). Increased survival may be due to a number of causes. Earlier diagnosis and improved therapy used in these patients are possibilities.

It is important to note that decreasing the potential exposure by limiting the duration of employment on the process only serves to increase the number at risk.

IV. Environmental Fate and Effect

In 1965, Takemura et al. (6) published their results of a survey of the pollution of the Sumida River in Tokyo, Japan. Concentrations of aromatic amines found in the water ranged between 205 to 562 ug/l. Concentrations of the carcinogenic amine, benzidine, identified by chromatography and determined quantitatively by the chloramine-T method, ranged from a maximum of 233 ug/l to a minimum of 82 ug/l. The source of these pollutants was suspected to be from wastes discharged by dye and pigment factories along the river. It was considered that the aromatic amines in the river were not necessarily discharged directly in the factories' wastewater but possibly were produced by the reduction of azo-dyes in the wastes by H_2S or SO_2 in the river water. Laboratory demonstrations showed that if H_2S

is bubbled for a few minutes through a pure azo dye solution, aromatic amines are liberated from the azo dyes.

To determine whether benzidine persists in natural waters for long periods of time or degrades relatively rapidly, the Great Lakes Laboratory experimentally studied the degradation of benzidine in lake water under laboratory conditions (34). Lake Erie water was obtained from the inlet of the Buffalo city water treatrent plant. Sample aliquots were spiked with initial benzidine concentrations of 1, 2 or 5 ug/l. The samples were treated under several conditions: 1 or 2 mg/l available chlorine, stirred, aerated and undisturbed and all samples were shielded from light. Results of the degradation of benzidine under various conditions have been tabulated in Table 5.

Table 5

Degradation of Benzidine Under Varying Conditions

BENZIDINE IN CHLORINATED LAKE WATER (1 mg/l Cl)

TIME (hrs.)	BENZ. CONC. in SOLN. A (ug/l)	BENZ. CONC. in SOLN. B ug/l)	BENZ. CONC. in SOLN. C (ug/l)
1/2	0.84	1.90	4.42
4	0.48	1.17	4.06
12	0.10	1.17	2.17
24	N.D.	N.D.	N.D.
48	-	-	-
72	-	-	-

BENZIDINE IN CHLORINATED LAKE WATER (2 mg/l Cl)

TIME (hrs.)	BENZ. CONC. in SOLN. A (ug/l)	BENZ. CONC. in SOLN. B ug/l)	BENZ. CONC. in SOLN. C (ug/l)
1/2	0.79	1.73	4.37
4	0.39	1.09	4.10
12	0.04	0.31	1.87
24	N.D.	N.D.	N.D.
48	-	-	-
72	-	-	-

where: Soln. A = Solution with initial benzidine concentration of 1 ug/l.

Soln. B = Solution with initial benzidine concentration of 2 ug/l.

Soln. C = Solution with initial benzidine concentration of 5 ug/l.

N.D. = Not detectable (<0.2 ug/l).

Table 5 (Cont.)

BENZIDINE IN AERATED LAKE WATER

TIME (hrs.)	BENZ. CONC. in SOLN. A (ug/l)	BENZ. CONC. in SOLN. B ug/l)	BENZ. CONC. in SOLN. C (ug/l)
1/2	0.83	1.98	4.61
4	0.69	1.42	4.27
12	0.31	0.72	2.01
24	N.D.	N.D.	N.D.
48	-	-	-
72	-	-	-

BENZIDINE IN STIRRED LAKE WATER

TIME (hrs.)	BENZ. CONC. in SOLN. A (ug/l)	BENZ. CONC. in SOLN. B ug/l)	BENZ. CONC. in SOLN. C (ug/l)
1/2	0.90	1.87	4.70
4	0.70	1.37	4.21
12	0.48	0.80	2.15
24	N.D.	N.D.	N.D.
48	-	-	-
72	-	-	-

BENZIDINE IN UNDISTURBED LAKE WATER

TIME (hrs.)	BENZ. CONC. in SOLN. A (ug/l)	BENZ. CONC. in SOLN. B ug/l)	BENZ. CONC. in SOLN. C (ug/l)
1/2	0.89	1.85	4.81
4	0.71	1.45	4.03
12	0.47	0.76	2.07
24	0.02	N.D.	N.D.
48	N.D.	-	-
72	-	-	-

The results show that the decay of benzidine in lake water is relatively rapid. At all three concentrations, regardless of the system, benzidine was at or below the detection limits after 24 hours. However, the experiments did not indicate the by-products, kinetics and/or mechanisms of this decay.

A further investigation by the Great Lakes Laboratory (57) determined that the rate of degradation of benzidine in natural waters was near a first-order reaction with a rate constant of 0.175 per hour.

To further evaluate the fate of benzidine in the aqueous environment and to define the role of sunlight, a series of studies were conducted to determine the photodegradation effect by light (58). The photodegradation effect was qualitatively determined by comparing the observed and calculated concentrations at various times during the reaction. The calculated values were determined by using the first-order decay equation and a rate constant of 0.175 per hour as previously determined (57). Results of the experiment have been tabulated in Table 6.

Table 6

PHOTODEGRADATION OF BENZIDINE IN WATER

Series A

Reaction Time (hours)	Benzidine Concentration Observed	Calculated
0	1.0 mg/l	1.0 mg/l
0.5	0.73	0.91
1.5	0.66	0.77
4.0	0.45	0.50
12.0	0.087	0.12
24.0	ND	ND
27.0	ND	ND

Series B

0	10.00 mg/l	10.00 mg/l
0.5	8.70	9.16
1.5	8.05	7.69
4.0	6.45	4.96
12.0	4.5	1.22
24.0	2.49	1.50
29.0	1.80	0.06
48.0	0.50	ND
53.0	0.29	ND
72.0	0.018	ND
77.0	0.018	ND

Series C

0	100.00 mg/l	100.00 mg/l
0.5	91.95	91.62
1.5	86.93	76.91
4.0	95.61	49.65
12.0	75.41	12.24
24.0	63.68	1.50
28.0	52.13	0.69
56.0	31.15	ND
80.0	22.92	ND

ND = Not Detectable (<0.2 ug/l)

For series A the observed values were always less than the calculated values, indicating that photodegradation enhanced the rate of degradation of benzidine. For series B and C, a reddish-brown material precipitated, serving as a light screen which diminished the rate of degradation. For the preliminary studies presented, the data indicate that for low concentrations of benzidine (less than 1 mg/l), photodegradation enhances the rate of degradation of benzidine in aqueous solutions.

However, in the photodegradation study (Table 6), the dependence of decay on the ambient water temperature was not studied. The mean temperature of the samples in the fadometer was 48°C. This temperature is atypically high of natural waters.

The biodegradation of benzidine was studied by Malaney et al. (35) and Lutin et al. (59) using Warburg respirometers which contained samples of activated sludge obtained from three different wastewater treatment plants. The studies conducted used sludge concentrations of 2500 and 5000 mg/l mixed liquor suspended solids (MLSS) and a benzidine concentration of 500 mg/l. The results showed that benzidine was inhibitory to bio-oxidation by each of the three sludges. The extent of removal of benzidine by bio-oxidation in an activated sludge aeration tank was found

to be insignificant at the concentration studied. It was postulated that benzidine may be removed partially or completely by adsorption. However, this mechanism was not investigated.

A similar study was conducted during 1973 by Ryckman et al. (60) using Warburg respirometers and two different types of sludges: An aniline acclimated sludge and a municipal wastewater treatment plant sludge. The sludge samples were spiked with two different concentration levels of benzidine, 1 and 5 mg/l. The results showed that benzidine appeared to be slightly inhibitory to both the aniline acclimated and non-acclimated municipal sludges. Still, both of the sludges showed rather large uptakes of benzidine during the 12-hour studies. Approximately 78 and 60 percent uptakes were noted for the acclimated and non-acclimated sludges, respectively for the samples with a concentration of 1 mg/l benzidine. For the samples containing 5 mg/l benzidine, uptakes of 64 and 50 percent were observed for the respective sludges. It was hypothesized that an adsorption mechanism was involved in removing the benzidine. However, no conclusive proof was obtained to either support or reject the hypothesis of an adsorption mechanism.

An investigation of the bio-oxidation of benzidine was conducted for SOCMA by International Hydronics, Inc. (61).

Studies were conducted to determine if benzidine can be biochemically oxidized at a concentration of 50 ug/l. The fate of benzidine was studied through a series of tests using acclimated and non-acclimated sludges. As interpreted by SOCMA, the tests produced the following results:

1) Benzidine can be biologically oxidized at a level of 50 ug/l. Oxidation of about 90 percent of the compound is accomplished.

2) Air oxidation of benzidine in a sterilized activated sludge will occur. About 30 percent of the benzidine concentration, 50 ug/l, can be reduced in this manner.

However, neither of these experiments demonstrated oxidation as such (for example, recovery of 14C carbon dioxide after treatment of labeled benzidine), but depended on measuring the decrease in benzidine across the system.

During 1975, Aquatic Toxicology Laboratory of Bionomics (62) conducted studies to determine the bioconcentration of 14C-benzidine in bluegill populations. The radiometeric method employed measured only 14C-residues. These residues were not identified to determine if the 14C was in the form of benzidine or a metabolite. During the six week

observation period following the introduction of bluegill to the test units, the cumulative mortality was 6 percent for fish exposed to 14C-benzidine at a mean water concentration of 191.02 ug/l, 8 percent for fish exposed to a mean water concentration of 98.07 ug/l, and 2 percent for the fish in the control unit. Bioconcentration factors of 38 times the mean concentration of 191.02 ug/l and 44 times the mean concentration of 98.07 ug/l were found in the edible portions of the bluegills after the 42 days of exposure.

Following the 42 days of benzidine exposure there was a 14-day depuration period to determine the rate and extent of the elimination of the 14C-residues. Within the first week of depuration, the edible portions of bluegill exposed to the high treatment level (191.02 ug/l) of 14C-benzidine eliminated 47 percent of the 14C-residue present at the end of exposure. By the end of the depuration period, 70 percent of these residues had been eliminated. Within seven days after commencing depuration, the bluegills exposed to the low treatment level (98.07 ug/l) of 14C-benzidine eliminated 42 percent of the 14C-residue present at the end of exposure. By the end of the depuration period, 73 percent of these 14C-residues had been eliminated from the edible portions of the exposed bluegills.

Bioconcentration studies show the edible portions of fish bioconcentrate 14C-residues from labeled benzidine to a factor of 44 times the water concentration, thus exhibiting an exposure route to man. However, following a 14 day depuration period, 73 percent of the bioconcentrated 14C-residues from labeled benzidine had been eliminated.

V. Criteria Formulation

Benzidine has not been studied extensively for toxicity to aquatic species since the main concern has been its carcinogenic activity in man. However, initial bioassays with benzidine have produced 96 hour TI_{50}'s of 2 mg/l for the fathead minnow and 5.0 for the emerald shiner (19) and 2.5, 4.35, and 7.4 mg/l for the red shiner, lake trout and rainbow trout respectively (18). A bioaccumulation study with benzidine has shown that edible tissue residues accumulate 44 times the ambient water level. Use of an 0.01 application factor applied to the 96 hour TL_{50} for the red shiner results in a level of 25 ug/l which would provide an ample margin of safety for aquatic life.

However, the principal objective in regulating benzidine under section 307(a) of the Federal Water Pollution Control Act is to minimize the risk of human exposure to benzidine which would result from its discharge to the navigable

waters in light of its proven carcinogenicity. See 38 Fed. Reg. 24343 (September 7, 1973). Accordingly, the recommended ambient water criterion is to provide adequate protection for human health as well as for aquatic organisms. It must minimize any unreasonable risk of human exposure.

Observations of the incidence of human bladder cancer due to occupational exposure have demonstrated benzidine to be a human carcinogen. To date no minimum effect level or maximum safe level has been established for humans. Extrapolation through mathematical modeling is one approach the Agency has used to determine levels of exposure which are likely to result in a low risk, or incidence, of cancer. The two most widely utilized models for this approach are the "one-hit" method (70) and the Mantel-Bryan method (64). In developing and applying these formulae, animal studies were used to experimentally demonstrate the relationship between various dose levels and the frequency of resultant tumors. Based on these experimental dose/effect data, it is possible to theoretically extrapolate the dose for low effect levels when a carcinogen is administered at a given daily dose over a lifetime.

There are several caveats which apply in using this type of mathematical modeling. First, this type of modeling is

not accepted by some sectors of the scientific community. It is not sufficiently advanced or proven as to justify use of this approach alone as the sole basis for establishing "safe" levels of exposure. If one is to have a high degree of confidence in predicting the likely dose/effect level, a larger statistical data base than that which is available should be utilized. However, the risk extrapolative methods may furnish an indication of risk levels associated with a particular exposure level or exposure levels which result in a predetermined level of risk, e.g., one cancer in a million of the exposed population.

With these qualifications in mind, each of the two mathematical models was applied to the three best available sets of dose/effect data (7, 33, 71). The calculations are set forth in Appendix 1, and are summarized in the following table.

Table 1 shows levels of benzidine which, based on a daily exposure over an entire lifetime, would produce no more than one cancer in one million population at risk at the 95 percent confidence limit:

Table 1

Observed Dose-Response Data	Extrapolative Method	
	One-Hit (70)	Mantel-Bryan (64)
Spitz (7)	0.056 ug/l	0.608 ug/l
Prokofjeva (33)	0.048 ug/l	0.212 ug/l
Vesselinovitch (71)	0.061 ug/l	0.843 ug/l

The Agency does not possess data as to the presence of benzidine in waters at the levels indicated in the above chart. Such measuring as has been done fails to show benzidine in the water columns at detectable levels. As noted earlier, the Agency lacks evidence of direct human exposure to benzidine from drinking water on the aquatic environment.

Under these circumstances, and in light of the previously expressed caveats concerning use of mathematical modeling, the Agency has concluded that a level of 0.1 ug/l, which approximates the low side of the middle range of the exposure levels reflected in the mathematical modeling exercises (0.05-0.84 ug/l), should provide the requisite margin of safety for human health as well as for aquatic organisms. This level is recommended as the ambient water criterion.

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Appendix 1

Various mathematical models can be used for deriving a level from the experimental dose/effect data which will ensure a limit of no more than one cancer in one million ($1x10^{-6}$) of the human population at risk at a 95 percent confidence level. The mathematical models employed to calculate this level for benzidine were the Mantel-Bryan probit model (64) and the one-hit model (70).

The Mantel-Bryan probit model is based on the assumption that the susceptibility to the carcinogenic effect of a chemical is distributed log normally among the population, and that the incidence of cancer will decrease with decreasing dose at a rate of no less than one normal deviation (probit) per 10-fold decrease in dose.

The one-hit model assumes that a single molecule can cause cancer. If the molecules are distributed at random, the probability of inducing cancer, as corrected for repeated cancer production, is proportional to the dose.

The relationship of an observed response, P, at a known dose, d, is expressed by the equation:

$$P=1-e^{-kd}$$

where:

P=observed response
d=dose
k=constant, calculated from experimental dose-response data.

With the calculated constant, the dose level at the limit of one cancer per one million at risk can then be calculated.

The dose-carcinogenic response studies reviewed for extrapolation to calculate human dose levels at the limit of no more than one cancer per one million at risk were reported by Griswold, et al., (31) using Sprague-Dawley rats; Spitz, et al., (7) using Sherman rats; Prokofjeva (33) using C3HA mice; the experiment conducted by Spitz, et al., (7) with dogs as reported in Bonser, et al., (26); and the experiment by Vesselinovitch, et al. (71), using B6C3F mice.

In the work reported by Griswold, et al., (31) a total of ten female Sprague-Dawley rats were orally administered benzidine for a period of 30 days to evaluate activity in causing breast cancer. The exquisite sensitivity of the mammary gland of the female Sprague-Dawley rats to certain chemical carcinogens is well defined in the literature, Huggins (66) and Shay, et al., (67). A serious statistical drawback associated with this study is the low number of

test animals employed. Because of the defined sensitivity of the female Sprague-Dawley rats and the statistically insignificant total of ten test animals used, the Griswold data was rejected for mathematical extrapolation.

Spitz's published conclusion (7) concerning their experiment with dogs was that no conclusion concerning the carcinogenicity of benzidine could be drawn from their reported one case of papillary carcinoma from seven test dogs. Six years after the publication of the test results from Spitz's experiment, Bonser, et al., in 1956 (26) reported a personal communication from Spitz stating that a total of three dogs developed papillomas and carcinomas of the bladder. However, because the necessary specific data has not been published, this experiment could not be used in the extrapolative methods.

The experiments reported by: Spitz, et al., (7); Prokofjeva (33); and Vesselinovitch (71), although not using random populations, are the best available data for calculation of human dose levels corresponding to no more than one cancer per one million at risk.

The dose administered to the experimental animals is first converted to an equivalent human dose. A conversion method is based upon the observation that for acute toxicity

of a number of anti-cancer agents, parameters such as the LD and the maximum tolerated dose are quantitatively proportional in mice, rats, hamsters, dogs, monkeys and man (65). This proportionality is based on a weight relationship, which is calculated by:

$$\text{human dose} = \left(\frac{\text{Average human weight}}{\text{Average animal weight}}\right)^{2/3} \times \text{animal dose}$$

In the work reported by Spitz, et al., (7) a total of 385 Sherman strain rats were injected subcutaneously in the back with a weekly dose of 15 mg of benzidine throughout the life of the animals until grossly obvious tumors appeared. Doses were not administered if the animals suffered loss of weight or obvious illness. Due to the high mortality of both the control and experimental series in the first 200 days of the experiment, only those animals which survived 200 or more days were counted as "at risk." Out of the original 385 animals injected with benzidine, a total of 206 survived 200 days or more. There were three tumor types in the test animals caused by the benzidine. These tumor types were: hepatomas; carcinomas arising in the specialized auditory sebaceous glands; and adenocarcinoma of the colon. A total of 100 test animals produced tumors of the three indicated types with an average incubation time of 222 days.

The administered dose of benzidine was 15 mg per week or 2.1 mg/day. The average weight of the experimental animals

was 265 gm, while the reported average weight of a human is 70kg (68). Calculating the equivalent human dose according to the 2/3 power of the weight ratio, as previously described, is demonstrated in the following equation:

$$\text{equivalent human dose} = \left(\frac{\text{Average human weight}}{\text{Average rat weight}}\right)^{2/3} \times \text{dose in animals}$$

$$\text{human dose} = \left(\frac{70\ \text{Kg}}{0.265\ \text{Kg}}\right)^{2/3} \times 2.14\ \text{mg/day}$$

$$\text{human dose} = 88.16\ \text{mg/day}$$

Assuming no sources of benzidine intake other than the average intake of two liters of water per person per day (69), this dose corresponds to a concentration of 44.08 mg/l in water.

The incidence of tumors from benzidine was 0.480 (48%), with the upper 95 percent confidence limit being 0.543 (54.3%). According to the one hit model, with a dose (d) of 44.08 mg/l, a response (P) of 0.543 (54.3%) at the upper 95 percent confidence limit, the constant k is calculated as:

$$P = 1 - e^{-kd}$$

$$k = \frac{-\ln(1 - P)}{d} = \frac{-\ln(0.457)}{44.08} = \frac{0.7831}{44.08}$$

$$k = 1.78 \times 10^{-2}$$

Deriving a concentration which, at the 95% confidence limit, will result in no more than one cancer per million of the population at risk (1×10^{-6}) yields:

$$d = \frac{-\ln(1 - P)}{k} = \frac{-\ln(0.999999)}{1.78 \times 10^{-2}} = \frac{10^{-6}}{1.78 \times 10^{-2}}$$

$$d = 5.62 \times 10^{-5}\ \text{mg/l}$$

At a 95 percent confidence level, the limit of one cancer per million at risk corresponds to a concentration of 0.056 ug/l, as extrapolated from the Spitz data calculated by the one-hit model.

Using the Mantel-Bryan method, a risk level of one in one million at risk is equivalent to a probit of -4.75. While the upper 95 percent confidence limit of the observed response corresponds to a probit of 0.108. Therefore, extrapolating from the experimental response of 0.543 (54.3%) to a response of no more than one cancer per million at risk corresponds to a probit change from 0.108 to -4.75 equal to -4.86.

According to the ten-fold reduction in dose per one probit, a probit change of -4.86 corresponds to a concentration of benzidine which will produce no more than one cancer per million in the population at risk of:

$$\begin{aligned} \text{concentration} &= 44.08 \text{ mg/l} \times 10^{-4.86} \\ &= 44.08 \text{ mg/l} \times (1.38 \times 10^{-5}) \\ &= 6.08 \times 10^{-4} \text{ mg/l} \\ &= 0.608 \text{ ug/l} \end{aligned}$$

At the 95 percent confidence limit, the concentration which will result in no more than one cancer per million at risk is equal to 0.608 ug/l, as extrapolated by the Mantel-Bryan method using the Spitz data.

In the experiment reported by Prokofjeva, a total of 181 C3HA mice were given subcutaneous injections of benzidine at a single weekly dose of 6 mg per mouse over a period of 8-13 months. Hepatomas appeared in 31 of 46 surviving test mice with the first occurence being within 15 to 16 months. In a defined control population, a one percent hepatoma frequency was observed. The administered dose of benzidine (6 mg/week) was equal to 0.856 mg/day. Calculating the equivalent human dose, assuming the average weights of 30 grams for an adult C3HA mouse (68) and 70 Kg for an adult human (68), the following dose is:

$$\text{equivalent human dose} = \left(\frac{70\ \text{Kg}}{0.03\ \text{Kg}}\right)^{2/3} \times 0.856\ \text{mg/day}$$

$$\text{human dose} = 150.48\ \text{mg/day}$$

Assuming no sources of benzidine intake other than the average intake of two liters of water per person per day (69), this dose corresponds to a concentration of 75.24 mg/l in water.

The incidence of tumors from benzidine was 0.674 (67.4%) with the upper 95 percent confidence limit equal to 0.788 (78.8%). Calculating the dose corresponding to the level of

no more than one cancer per one million at risk using the one-hit model, the following level is calculated:

Solving for k according to the Prokofjeva data:

$$P = 1 - e^{-kd}$$

$$k = \frac{-\ln(1 - 0.788)}{75.24 \text{ mg/l}} = \frac{1.55117}{75.24}$$

$$k = 2.06 \times 10^{-2}$$

Solving for the dose (d) when the response (P) is no more than one cancer per million at risk (10^{-6}):

$$d = \frac{-\ln(1 - 10^{-6})}{2.06 \times 10^{-2}} = \frac{10^{-6}}{2.06 \times 10^{-2}}$$

$$d = 4.85 \times 10^{-5} \text{ mg/l}$$

At the 95 percent confidence level a limit of no more than one cancer in one million at risk corresponds to a concentration level of 0.048 ug/l as calculated by the one-hit theory based upon the Prokofjeva data..

The corresponding probit for 0.788 (78.8%), which is the 95 percent confidence limit of the observed response, equals 0.80 probits. The probit change from an observed incidence of 0.788 (78.8%) to one in one million at risk equals -5.55, a probit change from 0.80 to -4.75.

According to the ten-fold reduction in dose per one probit change, a probit change of -5.55 corresponds to a concentration of benzidine which will produce no more than one cancer per million in the population at risk of:

$$\text{concentration} = 75.25 \text{ mg/l} \times 10^{-5.55}$$

$$= 75.24 \text{ mg/l} \times (2.82 \times 10^{-6})$$

$$= 2.12 \times 10^{-4} \text{ mg/l}$$

$$\text{concentration} = 0.212 \text{ ug/l}$$

At the 95 percent confidence limit, the concentration which will result in no more than one cancer per million at risk is equal to 0.212 ug/l, as extrapolated by the Mantel-Bryan method using the Prokofjeva data.

The study by Vesselinovitch, et al. (71), consisted of an integrated series of studies to assess the carcinogenicity of benzidine dihydrochloride in mice. In one carcinogenicity study, 200 six-week old B6C3F mice were given benzidine dihydrochloride intermittently by stomach intubation, twice weekly, in the amounts of 0.5 or 1.0 mg/mouse at each intubation for 84 weeks. All animals were killed at 90 weeks of age, at which time their tumor incidence was evaluated. Benzidine-treated mice developed liver tumors, Harderian gland tumors, lung adenomas, and lymphoreticular tumors. A total of 124 of the indicated tumors were reported for both dose levels. The average dose level is calculated to be 0.428 mg/day/mouse. Calculating the equivalent human dose, assuming the average weights of 30 grams for an adult B6C3F mouse (68) and 70 kg for an adult human (68), the following dose is:

$$\text{equivalent human dose} = \left(\frac{70\ \text{Kg}}{0.03\ \text{Kg}}\right)^{2/3} \times 0.428\ \text{mg/day}$$

$$\text{human dose} = 75.24\ \text{mg/day}$$

Assuming no sources of benzidine intake other than the average intake of two liters of water per person per day

(69), this dose corresponds to a concentration of 37.62 mg/l in water.

The incidence of tumors from benzidine was 0.413 (41.3%) with the upper 95 percent confidence limit equal to 0.460. The dose corresponding to a level of no more than one cancer per one million at risk, using the one-hit model, is calculated as follows:

Solving for k according to the Vesselinovitch data:

$$k = \frac{-\ln(1 - P)}{d} = \frac{-\ln(0.540)}{37.62} = \frac{0.61619}{37.62}$$

$$k = 1.64 \times 10^{-2}$$

Solving for the dose (d) when the response (P) is no more than one cancer per million at risk (10^{-6}):

$$d = \frac{-\ln(1 - 10^{-6})}{1.64 \times 10^{-2}} = \frac{10^{-6}}{1.64 \times 10^{-2}}$$

$$d = 6.10 \times 10^{-5} \text{ mg/l}$$

At the 95 percent confidence level a limit of no more than one cancer in one million at risk corresponds to a concentration level of 0.061 ug/l as calculated by the one-hit theory based upon the Vesselinovitch data.

The corresponding probit for 0.460 (46.0%), which is the upper 95 percent confidence limit of the observed response, equals -0.100. The probit change from an observed incidence of 0.460 (46.0%) to one in one million at risk equals -4.65, a probit change from -1.00 to -4.75.

According to the ten-fold reduction in dose per one probit change, a probit change of -4.65 corresponds to a concentration of benzidine which will produce no more than one cancer per million in the population at risk of:

$$\text{concentration} = 37.62 \text{ mg/l} \times 10^{-4.65}$$
$$= 37.62 \text{ mg/l} \times (2.24 \times 10^{-5})$$
$$= 8.43 \times 10^{-4} \text{ mg/l}$$
$$\text{concentration} = 0.843 \text{ ug/l}$$

At the upper 95 percent confidence limit, the concentration which will result in no more than one cancer per one million at risk is equal to 0.843 ug/l, as extrapolated by the Mantel-Bryan method using the Vesselinovitch data.

Table 7 presents a summary of the calculated concentrations which will result in no more than one cancer per million at the upper 95 percent confidence limit as extrapolated by the Mantel-Bryan and the one-hit methods, from dose-response data as reported by Spitz, et al., (7), Prokofjeva (33), and Vesselinovitch, et al. (71).

Table 7

Summary of Calculated Concentrations

Observed Dose-Response Data	Extrapolative Method	
	One-Hit (70)	Mantel-Bryan (64)
Spitz (7)	0.056 ug/l	0.608 ug/l
Prokofjeva (33)	0.048 ug/l	0.212 ug/l
Vesselinovitch (71)	0.061 ug/l	0.843 ug/l

GLOSSARY

Acutely toxic: Causing death or severe damage to an organism by poisoning during a brief exposure period, normally ninety-six hours or less.

Anadromous fishes: Fishes that spend a part of their lives in seas or lakes, but ascend rivers and streams at certain intervals to spawn. Examples are sturgeon, shad, salmon, trout, and striped bass.

Application factor: The ratio of the safe concentration to the lethal concentration as determined for potential aquatic pollutants administered to species of interest.

Bioaccumulation (Bioconcentration): The phenomenon wherein elements or compounds are stored in living organisms because elimination fails to match intake.

Carcinogenic: Producing Cancer.

Catadromous fishes: Fishes that feed and grow in fresh water, but return to the sea to spawn. The best example is the American eel.

Chronically toxic: Causing death or damage to an organism by poisoning during prolonged exposure, which, depending on the organism tested and the test conditions and purposes, may range from several days, to weeks, months, or years, or through a reproductive cycle.

EC50: The concentration at which a specified effect is observed under the test conditions in a specified time in fifty percent of the organisms tested. Examples of specified effects are hemorrhaging, decreased feeding, dilation of pupils, and altered swimming patterns.

Epilimnion: That region of a body of water that extends from the surface to the top of the thermocline and does not have a permanent temperature stratification.

Flow-through bioassay: An assay system in which aquatic species are exposed to toxicants in a constantly flowing system, and where the toxicant is replenished continuously or discontinuously.

Hardness (Water): The concentration of the polyvalent metallic ions dissolved in water. Unually it is reported as the equivalent concentration of calcium carbonate ($CaCO_3$).

Hyperplasia: Abnormal multiplication or increase in the number of normal cells in normal arrangement in a tissue.

Hypolimnion: The region of a body of water that extends from the bottom of the thermocline to the bottom of the water body and is essentially independent of most surface phenomena.

LC25: The concentration of a toxicant that is lethal (fatal) to twenty-five percent of the organisms tested under the test conditions in a specified time.

LC50: The concentration of a toxicant which is lethal (fatal) to fifty percent of the organisms tested under the test conditions in a specified time. It is virtually identical with TLm and TL50.

LD50: The dose of a toxicant that is lethal (fatal) to fifty percent of the organisms tested under the test conditions in a specified time. A dose is the quantity actually administered to the organism and is not identical with a concentration, which is the amount of toxicant in a unit of test medium rather than the amount ingested by or administered to the organism.

Liter (l): The volume occupied by one kilogram of water at a pressure of 760 mm of mercury and a temperature of 4 °C. A liter is 1.057 quart.

Methylmercury: Mercury which has been methylated, usually through some biological agent, such as bacteria.

Microgram per liter (ug/l): The concentration at which one millionth of a gram (one microgram) is contained in a volume of one liter. Where the density of solvent is equal to one, one ug/l is equivalent to one part per billion (ppb) or one microgram per kilogram (ug/kg).

Microgram per kilogram (ug/kg): The concentration at which one millionth of a gram (one microgram) is contained in a mass of one kilogram. A kilogram is 2.2046 pounds.

Milligram per kilogram (mg/kg): The concentration at which one thousandth of a gram (one milligram) is contained in a mass of one kilogram. A gram contains 1000 milligrams.

Milligram per liter (mg/l): The concentration at which one milligram is contained in a volume of one liter. Where the density of the solvent is equal to one, one mg/l is equivalent to one part per million (ppm) or one milligram per kilogram (mg/kg).

Milliliter (ml): A volume equal to one thousandth of a liter.

Nanogram per liter (ng/l): The concentration at which one billionth of a gram (one nanogram) is contained in a volume of one liter. Where the density of the solvent is equal to one, one ng/l is equivalent to one part per trillion or one nanogram per kilogram (ng/kg).

Neoplastic: Describing any new and abnormal growth, such as a tumor.

Part per million (ppm): A concentration in which one unit is contained in a total of a million units. Any units may be used (e.g., weight, volume) but in any given application identical units must be used (e.g., grams per million grams or liters per million liters). Where the density of the solvent is one, one part per million is equivalent to one milligram per liter.

Parts per thousand (o/oo): A concentration at which one unit is contained in a total of a thousand units. The rules for using this term are the same as those for parts per million. Normally, this term is used to specify the salinity of estuarine or sea waters.

Piscicide: A substance used for killing fish.

Static bioassay: A bioassay in which the toxicant is not renewed during the test.

Thermocline: That layer in a body of water where the temperature difference is greatest per unit of depth. It is the layer in which the drop in temperature is 1 °C. or greater per meter of depth.

TLm - Median Tolerance Limit: The concentration of a test material at which fifty percent of the test animals are able to survive under test conditions for a specified period of exposure. It is virtually synonymous with LC50 and TL50.

TL50: Synonymous with TLm and virtually synonymous with LC50.

Tumorigenic: Causing or producing tumors.